July

Week 1 (04/07/16 - 08/07/16)

PCR and restriction have to be carried out for achieving the documentation J04500-K1184000 that will be used for characterization of biobricks.
In order to test the promoter efficiency it is necessary to realize IPTG induction.
Solubility analysis allows us to determine protein location (Soluble or insoluble phase). Results of induction and solubility can be observed in SDS PAGE.

- MODULE 1: Plasmid extraction

R0011-K1362011
The lysozyme cassette extraction was performed taking into consideration several factors that were not considered in previous extractions such as the bacterial strain and how its genome can affect the extraction itself. Escherichia coli BL21 is a special strain that will express more protein, different from Escherichia coli DH5-alpha, which is most suitable for cloning and plasmid production.

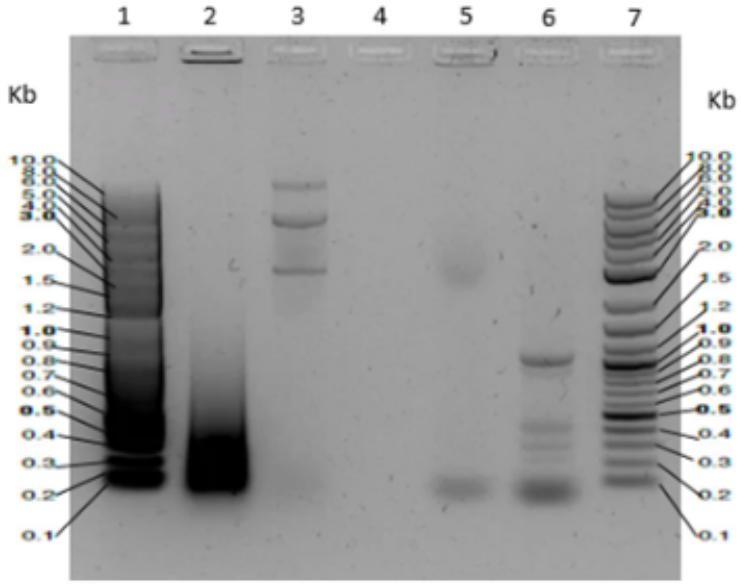

Figure 1. Electrophoresis of a plasmid digestion of J04500+K1184000 (30/06/2016) and a PCR product of R00λ and Killer Red expression cassette in a 0.8% agarose gel run in a 0.5X TBE buffer at 100 V for 50 minutes and stained with GelRed 0.3X. The PCR products of Killer Red and R00λ expression cassettes have an expected length of 1264 bp and 840 bp, respectively. **1)** Quick-Load Purple 2-log DNA ladder diluted 1:3 in MB grade water (NEB) (3 µL + 4 µL LB), **2)** J04500+K1184000 digestion (4.2.D) (5 µL + 4 µL LB), **3)** J04500+K1184000 digestion (β) (3 µL + 4 µL LB), **4)** Fourth well was skipped, **5)** R0011+K1362011 PCR (5 µL + 4 µL), **6)** J04500+K1184000 PCR (5 µL + 4 µL) **7)** Quick-Load Purple 2-log DNA ladder diluted 1:3 in MB grade water (NEB).

J04500-K118400
This extraction shows significant amounts of protein contamination and 5 DNA bands, which do not correspond to a normal plasmid extraction. This plasmid extraction was not reliable and should not be trusted for further experiments.

- MODULE 2: Polymerase Chain Reaction

Regarding results obtained after a PCR was carried out and documented by means of a gel electrophoresis, it can be clearly seen that there is a PCR product of 1264 bp in the sixth well (between the 1.0-1.2 kbp DNA ladder bands) corresponding to the KillerRed expression cassette.

- MODULE 3: Restriction

The restriction of J04500-K1184000 provided the expected three bands. The differentiating bands differ in 226 pb, which explains that both bands have a similar migration pattern in the gel.

The restriction of R0011-K1362011 was not successful. Problems with the enzyme are unlikely because the digestion of J04500-K1184000 gave the results of the design.

- MODULE 4: Recombinant protein expression / induction with IPTG (R0011-K1362011 and J04500-K1884000)

J04500-K1184000
The part has shown detectable fluorescence in high-copy plasmids. The SDS-PAGE analysis is still not conclusive.

R0011-K1362011
Lambda lysozyme causes cell wall degradation, then bacterial cell number does not increase after the protein accumulates, which results in a constant amount of expressed protein over time. Wild-Type LacI did not seem to have considerable effects on the expression, registering the protein until IPTG was added.

- MODULE 5: Solubility analysis

J04500-K1184000
Killer red cassette (J04500-K1184000) has an expected weight of 26.55 kDa. The protein is present on a larger amount in the insoluble phase, also is present in less quantity in the soluble phase.

R0011-K1362011
The lambda lysozyme cassette (R0011-K1362011) with an expected weight of 17.83 kDa, is present on a larger amount in the insoluble phase (in form of inclusion bodies). It shows almost no amount of soluble protein.

Summary:

The documentation of the cassette containing K1184000-J04500 showed that we successfully extracted, digested and ligated the plasmid.
In the biobrick Bba_K1362011 documentation we could observe that the plasmid was cut successfully and ran well in the electrophoresis gel.
Induction of KillerRed cassette was not successfully accomplished. On the other hand lambda lysozyme cassette showed the expected reaction with IPTG induction. After IPTG was added LacI had no more effects on the expression.
Although solubility analysis shows diffuse bands on both cassettes, protein of KillerRed is present on a larger amount on the insoluble phase. The protein of lambda lysozyme cassette is also present on a larger amount in the insoluble phase.

Week 2 (11/07/16 - 15/07/16)

Due to not conclusive results obtained on induction of J04500-K1184000 cassette, repetition of the processes regarding assembly was needed.
Referring to the R0011-K1362011 cassette, documentation will be performed by PCR and digestion. An IPTG induction is required in order to confirm the presence of the desired protein. Solubility analysis allows us to determine protein location (Soluble or insoluble phase).

- MODULE 1: Plasmid extraction

J04500 and K1184000
Two plasmid isoforms. These isoforms match the corresponding base pair length of the biobricks. A 2200 bp band approximately corresponds to the length of the J04500 promoter; also a band of 2800 bp migrated, indicating the presence of a plasmid with similar length to K1184000.

K1184000
A band which corresponds to the length of K1184000 is present. This plasmid extraction is reliable and should be trusted for further experiments.

- MODULE 2: Restriction

For the digestion, it can be seen that J04500 was digested with PstI and SpeI, expecting a linearized isoform with a length of 2068 bp and a short sequence of 222 bp.

K1184000 was cut with XbaI and PstI in order to get two fragments: one of approximately 2044 bp and another one of 749 bp. Since none of those fragments were achieved, it was decided to extract such plasmid again.

- MODULE 3: Transformation

Growth of transformed DH5α cells with BBa_K1184000 in LB+CAM medium. Two colonies were chosen and stria cultured, for further documentation of the biobrick.

- MODULE 4: Polymerase Chain Reaction

The lysozyme cassette PCR band was expected to weigh 836 pb according to the in silico PCR . However, the darkest bands do not correspond to the desired weight and the expected band is quite thin.

- MODULE 5: Induction and Solubility analysis

The presence of lysozyme can be determined because a band appears between 15-20 kDa, and the expected weight of the lysozyme corresponds to 17.83 kDa. It can be seen a gradient reaching its peak at well 4, which means that the protein production increased the first 2 hours and then it decreased. This behaviour is explained by the excessive production of lysozyme that triggers the death of cells, which in consequence, decreases the lysozyme production. However, the solubility analysis was not conclusive.

Summary:
Documentation of the cassette containing R0011+K1362011 was accomplished. Digestion and PCR both show the expected bands.
Regarding the cassette assembly (K1184000 + J04500), as the digestion of BBa_K1184000 with XbaI and PstI failed, NcoI was used to prove the presence of the desired plasmid. When this test did not show the expected band, the transformation and extraction of the part directly from the plate were made. The 2 parts of the cassette were digested, with J04500 acting as the plasmid backbone (SpeI and PstI) and K1184000 as the part to be inserted (XbaI and PstI). Purification was achieved for further assembly of the cassette.

Week 3 (18/07/16 - 22/07/16)

To achieve satisfactory results regarding the documentation of R0011-K1362011 and J04500-K1184000 biobricks, they were ligated and transformed.
Induction & solubility assays were performed. Also, a PCR was carried in order to amplify the cassettes and to prove its presence.

- MODULE 1: Transformation of ligation

J04500 and K1184000
Both transformations showed 4 CFU in LB + CAM mediums, which indicates that the transformed cells have resistance against the antibiotic employed. These results may represent that the cassette was correctly introduced to the cells.

- MODULE 2: Polymerase Chain Reaction of J04500 and K1184000

PCR was not successful

- MODULE 3: Induction and Solubility analysis

R0011-K1362011
The SDS page Gel shows faded bands in the proximity of the expected weight of the ligated protein R0011-K1362011 that get more intense in relation with time, which can provide evidence that the protein was correctly induced. Better resolution is still needed but is not indispensable to continue the characterization process.

Summary:
We can conclude, that given the expected results of our procedures and the ones obtained, we have a positive outcome, even though our transformed cells didn't grow in a big quantity, we can expect that the cassette assembly of BBa_J04500 coupled to BBa_K1184000 is present on our cells.
Regarding the SDS gel, we can see that the protein is present, and the negative and BL21 controls assure us that the other proteins that might have an approximate weight to ours are present, but we can still observe that the presence of the R0011-K1362011 increased with time. Considering this, we can now proceed to further protocols in order to characterize the desired protein.

Week 4 (25/07/16 - 29/07/16)

R0011-K1362011 and J04500-K1184000 induction and documentation

MODULE 1: Miniprep plasmid extraction

MODULE 2: Enzymatic restriction of KillerRed cassette (J04500-K1184000)

MODULE 3: Induction

MODULE 4 :Solubility analysis

Summary:
The 25 kDa ladder did not resolve correctly, causing inconsistencies on the location of the protein of interest with a molecular weight of 26.55kDa. The band immediately above the ladder shows no induction whatsoever. The next band shows increased intensity with time, which could be the protein of interest, nevertheless the migration pattern is too far from the 25kDa. The gel is insufficient but the restriction shows that previous ligations are correct, then the induction conditions should be checked. R0011-K1362011 and J04500- K1184000 induction and documentation